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HLA-DR-restricted T cell lines from newly diagnosed type 1 diabetic patients specific for insulinoma and normal islet beta cell proteins: lack of reactivity to glutamic acid decarboxylase

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SUMMARY

T cells reacting with pancreatic islet beta cell proteins play a pivotal role in the pathogenesis of type 1 diabetes in experimental animal models and man, although the islet cell autoantigens against which these T cells are directed remain to be characterized. We have previously shown the presence of disease-related antigens residing in the transplantable RIN insulinoma membranes which are recognized by T cells from diabetic NOD mice. We now report on the establishment of $CD4^+$, T cell lines reacting with insulinoma membranes from six newly diagnosed type 1 diabetic patients. Detailed examination of T cell lines from two patients revealed that both the lines continued to react with normal islet cell proteins and, interestingly, were also stimulated by antigens present in brain microsomes. The two T cell lines showed reactivity with different molecular weight proteins of the insulinoma membranes and both the lines were histocompatibility-linked antigen (HLA)-DR restricted. Although the insulinoma membrane preparation is known to contain glutamic acid decarboxylase (GAD), none of the six T cell lines proliferates in response to purified GAD. These T cell lines will be valuable in characterizing novel islet beta cell antigens which are likely to be implicated in type 1 diabetes.

Keywords T cells RIN insulinoma islet beta cells glutamic acid decarboxylase

INTRODUCTION

In type 1 (insulin dependent) diabetes mellitus (IDDM), the destruction of insulin producing pancreatic beta cells is believed to be mediated by autoreactive T cells [1]. In experimental animal models of type 1 diabetes such as the NOD mouse, a large body of evidence based upon the nature of the insulitis lesion in the pancreas, together with studies on cloned populations of T cells which transfer the disease in an adoptive transfer model, indicate a pivotal role for T cells in the pathogenesis of disease. In human disease, islet cell autoantibodies (ICA) are also present, sometimes well before the clinical onset of disease, but are unlikely to be involved in the destruction of islet beta cells. However, ICA represent good markers of disease activity. The list of islet cell antigens recognized by ICA is diverse [2] and usually includes antibodies to a 64-kD protein, now known to be glutamic acid decarboxylase (GAD) [3]. In contrast, only a few islet cell antigens recognized by T cells in type 1 diabetic patients have been identified; these include insulin, GAD and an uncharacterized 38-kD secretory granule protein [4–7].

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T cell reactivity to GAD in the NOD mouse has recently been shown to be important in the initiation and maintainance of autoimmune reactivity to islet beta cells, since induction of tolerance to GAD early in disease abrogates all subsequent insulitis and beta cell destruction [8,9]. Furthermore, considerable evidence has accumulated on the multiplicty of islet cell antigens recognized by T cells in the NOD animals [10,11]. These antigens remain uncharacterized. Our earlier studies showed the presence of disease-related antigens residing in the transplantable RIN insulinoma membranes that stimulated T cells from the unprimed NOD animals [10]. There have also been other detailed studies showing the presence of novel, early disease-related autoantigens in insulinoma tissues recognized by T cells from young NOD animals [11]. Indeed, the antigens recognized by disease-inducing, diabetogenic T cell clones have also been localized to insulinoma secretory granules [12]. Thus insulinoma tissue appears to contain a number of important, but so far uncharacterized antigens relevant to the disease-inducing function of T cells in type 1 diabetes.

Peripheral-blood-derived T cells from IDDM patients proliferate in the presence of insulinoma membranes [13,14] and long-term T cell clones have been established from these

patients that are either histocompatibility-linked antigen (HLA)-DR1 restricted [13] or HLA-DQ restricted [14]. One of the DR1 restricted T cell clones recognizes a 38-kD antigen [6] while others have been shown to react with different fractions of insulinoma granule membranes [15]. We report on the establishment of six antigen specific T cell lines to the transplantable RIN insulinoma membranes derived from newly diagnosed type 1 diabetic patients. Two of the T cell lines studied in detail show reactivity to different molecular weight insulinoma membrane proteins. Although GAD is present in the insulinoma membranes, none of the six T cell lines proliferate in response to purified GAD.

MATERIALS AND METHODS

Antibodies, reagents and cytokines

Antibodies used for typing CD4 and CD8 were obtained from Becton Dickinson (Oxford, UK) (Simultest CD4/CD8). The HLA class I (w6/32) and class II tissue culture supernatants containing MoAbs (HLA-DR (L243), and HLA-DQw1 (4D4) and HLA-DQw2 (C1A2 and LY6)) for characterization of HLA restriction elements of the antigen specific T cell lines were obtained from Dr Delores Schendel (Munich, Germany).

Lymphocult as a source of IL-2 was obtained from Biotest (Solihull, UK) and human rIL-4 from Genzyme (Cambridge, MA). Leucoagglutinin-PHA (Sigma, Poole, UK) was used at 1–10 μg/ml.

Patients and healthy blood donors.

Peripheral blood from six Caucasian, newly diagnosed IDDM patients was collected after informed consent from the patients and ethical approval from the institution; blood was collected either when the patients were first seen at the clinic (patients M.G. and K.W.) or within the first 2 to 4 weeks of clinical diagnosis (Table 1). All patients were placed on insulin therapy at the initial consultation. ICA antibodies were measured by immunofluorescence on unfixed, 5 μm cryostat sections of blood group O human pancreas. In the 111th Immunology and Diabetes Workshop on ICA proficiency, this assay performed with 100% specificity. The GAD antibody levels were measured by immunoprecipitation assay employing *in vitro* transcription and translation of ^{35}S-methionine-labelled human GAD65 [16]. This assay has been validated in the Second International GAD antibody workshop (laboratory number 52), with a sensitivity of 87% and a specificity of 91%. The results for both antibodies are reported as positive or negative. Typing for HLA class II DR and DQ antigens was determined either serologically by the micro-lymphocytotoxicity technique or by polymerase chain reaction-sequence specific primers (PCR-SSP) [17]. Peripheral blood was also obtained from two normal individuals (J.B. and E.H.) with no family history of IDDM; the two normals were as closely matched as possible for HLA class II to those of patients M.R. and H.S. whose T cell responses were characterized in this study (Table 1).

Crude membrane fraction of rat insulinoma tissue and normal rat islet cells.

Crude membranes from a transplantable RIN tumour were prepared by a modification of the previously described procedure [18] by centrifugation of the post nuclear supernatant from homogenized tumour cells at 48 000 ***g*** for 20 min. Normal rat pancreatic islets were purified by hand picking following collagenase digestion and cytoplasmic protein and microsomal membrane prepared by freeze-thaw lysis and homogenization in ice-cold phosphate-buffered saline containing a cocktail of protease inhibitors (100 μg/ml phenylmethylsulphonylfluoride, 100 μg/ml aprotinin, 10 μg/ml leupeptin and 10 μg/ml pepstatin) and differential centrifugation [10]. The washed pelleted microsomes were stored aliquoted at −70°C. Protein concentration was determined by Pierce assay. Microsomes from other rat tissues were similarly prepared, as described above for the RIN tumour. Human thyroid and liver microsomes were prepared from frozen tissue by homogenization in a polytron homogenizer and differential centrifugation, as described previously [19]. Crude membranes from RIN tumour were fractionated by SDS–PAGE and 15 different 0·5 cm strips containing different molecular weight proteins were electroeluted [6].

For T cell proliferative activity, all microsomal proteins were used at a final concentration of 30 μg/ml and the electroeluted proteins were used at a final concentration of 10 μg/ml. All antigen preparations were irradiated before cell culture for 100 Gy (0·01 Gy = 1 rad) using a cell irradiator (Gammacell 1000, Vinten Instruments, Ontario, Canada).

Table 1. HLA class II antigens and islet beta cell autoantibodies in newly diagnosed IDDM patients used for generation of T cell lines and normal controls

	Age/sex	ICA	GAD antibody	MHC HLA class II
IDDM patient				
M.R.	46/F	+ve	+ve	DR3,4; DR 52,53; DQ2,7
H.S.	30/M	+ve	+ve	DR1,7; DR53; DQ1
K.C.	24/M	+ve	−ve	DR3; DR52; DQ2
M.G.	31/F	−ve	+ve	DR3,4; DR52,53; DQ2,8
K.W.	29/F	+ve	+ve	DR3,4; DR52,53; DQ2,7
T.R.	26/M	−ve	+ve	DR4,14; DR53; DQ7,8
Normal controls				
E.H.	26/F	−ve	−ve	DR4,17; DR2,7
J.B.	60/F	−ve	−ve	DR1,7; DQ1,2

Purified porcine GAD

Highly purified porcine GAD prepared from pig cerebellum, resuspended at 500 μg/ml and dialysed against RPMI 1640 medium, was a gift from Dr Josef Endl (Penzberg, Germany). The proportion of GAD65 and GAD67 isoform in this preparation of GAD is not known. For generating antigen-specific human T cell lines and clones from IDDM patients, this preparation of GAD is used at 5–10 μg/ml [20]. In cultures of RIN antigen specific T cell lines, the porcine GAD was used at 3 and 10 μg/ml.

Establishment of antigen specific T cell lines

Peripheral blood leucocytes (PBL) were isolated from peripheral blood by density gradient centrifugation as described [21]. As a source of APC, PBL at 5×10^6 ml were irradiated at 30 Gy and stored frozen in liquid N_2. For the generation of T cell lines, 10^6 PBL in 1 ml complete medium (RPMI 1640 medium, containing 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin supplemented with 10% heat inactivated AB^+ human serum) were cultured with 30 μg/ml RIN insulinoma microsomal membrane in 24-well microtitre plates at 37°C in the presence 5% CO_2 in air. After 3 to 4 days in culture, 100 μl lymphocult was added to the cultures as a source of IL-2. After 10 days culture, the cells were restimulated with RIN antigen and irradiated autologous APC and cultured for an additional 7 days. At this stage, after two rounds of antigen stimulation, T cell lines were established.

T cell proliferation assay

T cell lines or clones at 10^4 cells/well in 96-well microtitre plates were incubated with irradiated, autologous APC (2×10^5 cells/well) in complete medium supplemented with different concentrations of RIN antigen, IL-2 or PHA and incubated for 55–60 h at 37°C with 5% CO_2 in a humidified incubator. The cells were pulsed with 0·5 μCi ^{3}H-thymidine per well and cultures continued for an additional 18 h before harvesting and counting in a beta plate counter (Packard). MHC restriction specificity was determined by blocking antigen or PHA-induced proliferation in the presence of MHC class I and class II allele specific MoAbs. All the MoAbs were first tested for cytotoxicity on the T cell lines in PHA-induced proliferation. The dilutions of the MoAb culture supernatants selected were those that showed no cytotoxic effects. Different dilutions of MoAb supernatants were added to cultures, as described above for proliferation experiments.

RESULTS

Generation and characterization of RIN antigen specific T cell lines

T cell lines to RIN microsomal membrane proteins were successfully generated from the peripheral blood of six newly diagnosed IDDM patients using autologous, irradiated PBL as a source of APC. Attempts to generate T cell lines from PBL of two normal individuals with no family history of IDDM, using the same protocol for generating lines from diabetic patients, were not successful.

All the T cell lines derived from IDDM patients showed low background responses to autologous APC without pulsed antigen whilst strong proliferative responses were apparent in the presence of antigen, PHA or IL-2 (not shown). Two of the lines, H.S. and M.R., where a plentiful supply of APC were readily available and which also exhibited good growth characteristics, were studied in detail. Double staining of M.R. and H.S. T cell lines by FACS analysis showed the majority of cells (> 90%) to be CD4 single positive cells with 5·4% and 0·6% CD8 single positive cells respectively; only the M.R. line comprised 1·7% CD4/CD8 double positive cells (not shown).

Dose response of M.R. and H.S. T cell lines

The two T cell lines showed typical responses to different doses of the RIN microsomal membrane proteins, with maximal responses at 30 μg/ml antigen (Fig. 1). The lines were subsequently stimulated with this optimal dose antigen.

Reactivity of M.R. and H.S. T cell lines with normal rat islet proteins

To confirm that the T cell lines were recognizing islet cell proteins rather than tumour antigens of the RIN insulinoma, T cell reactivity was determined to cytoplasmic and microsomal membrane proteins derived from normal rat islets. Both the T cell lines proliferated to the normal rat islet derived proteins, with maximal responses at 30 μg/ml for both the cytoplasmic and microsomal proteins (Fig. 2, M.R.; H.S. not shown).

Reactivity of M.R. and H.S. T cell lines to other rat and human tissue microsomal proteins

To determine if the antigen(s) recognized by the two RIN antigen specific T cell lines were islet specific, their proliferative response to a variety of rat and human tissues was ascertained. Neither of the two T cell lines showed any significant stimulation with human liver, human thyroid, rat kidney or rat stomach microsomes (Fig. 3, M.R.; H.S. not shown). In contrast, rat brain microsomes derived from cerebrum, cerebellum and cortex led to marked proliferation of both the lines, with the cortex microsomes being the most potent for both the lines (Fig. 3, M.R.; H.S. not shown). Significant proliferative activity was also observed with microsomes derived from rat hypothalamus, although we cannot be certain of contamination of brain tissue in the hypothalamus preparations (Fig. 3).

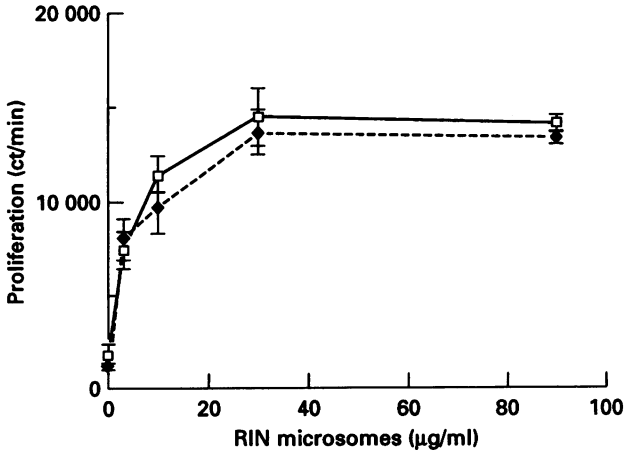

Fig. 1. Proliferative responses of M.R. (- - -) and H.S. (—) T cell lines to varying doses of RIN microsomal antigen. The results of ^{3}H-thymidine incorporation are shown and are expressed as mean ± s.e.

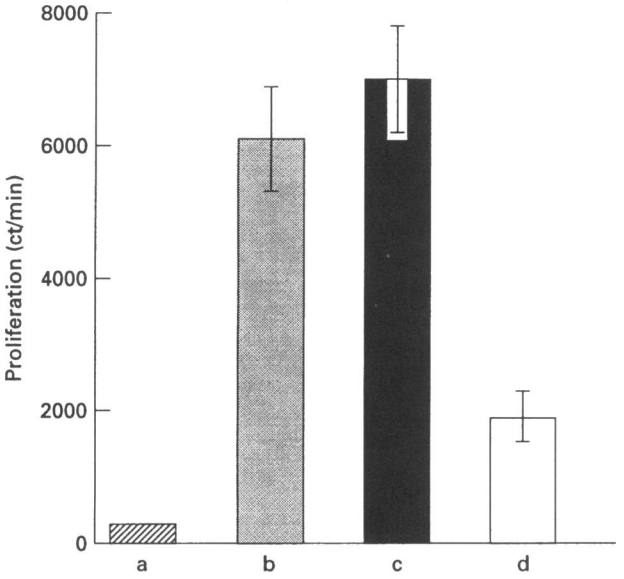

Fig. 2. Reactivity of the M.R. T cell line to normal rat islet proteins. Proliferative responses in the presence of (a) complete medium, (b) RIN microsomal membranes, (c) rat islet cytoplasmic extract, and (d) rat islet microsomes are shown. Each antigen was used at 30 μg/ml.

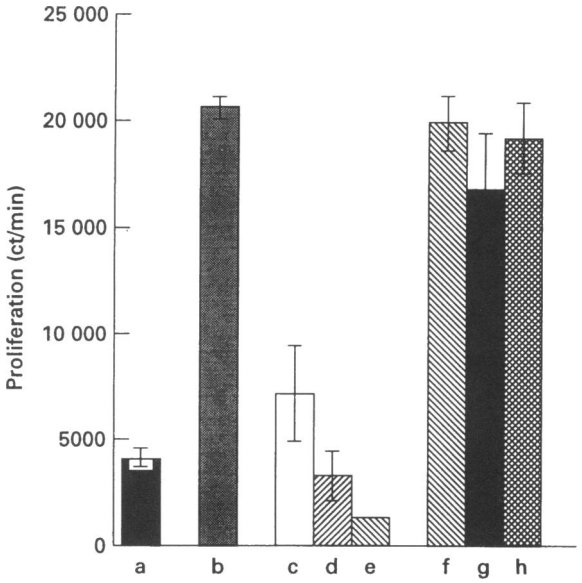

Fig. 4. Inhibition of proliferation of H.S. T cell line to RIN microsomes using a HLA-DR specific MoAb (L243) and HLA-DQw1 specific MoAb (4D4). T cells were cultured with irradiated APC and RIN microsomes, in the presence of MoAb containing tissue culture supernatant, added at different dilutions. As controls, responses to (a) complete medium, and (b) RIN microsomes in the absence of any MoAb are shown. T cell proliferation in the presence of L243 (c to e) and 4D4 (f to h) is shown. The final dilution of the MoAb containing tissue culture supernatant was 24-fold (c and f), 12-fold (d and g) and 6-fold (e and h).

HLA allele specific MoAb blocking of RIN antigen-induced proliferation

The HLA class II restriction elements of the two RIN antigen-specific T cell lines were examined by inhibition of antigen induced proliferation with HLA-DR (L243), and HLA-DQw1 (4D4) and HLA-DQw2 (C1A2 and LY6) MoAbs. Pilot experiments with these MoAbs in PHA-induced proliferation of the two T cell lines did not reveal any non-specific cytotoxic effects of the MoAbs on the T cells. HLA-DR MoAb was highly effective in inhibiting RIN antigen-induced proliferation of both the M.R. and H.S. T cell lines. The DQ MoAbs were used individually depending on the genotype of the patient. Addition of HLA-DQw1 specific MoAb on H.S. T cell line (Fig. 4) and HLA-DQw2 specific MoAb on M.R. T cell line (not shown) did not lead to any significant inhibition of proliferation. Anti-class I MoAb (w6/32) did not also show any inhibitory effects on antigen-induced proliferation of M.R. and H.S. lines (not shown).

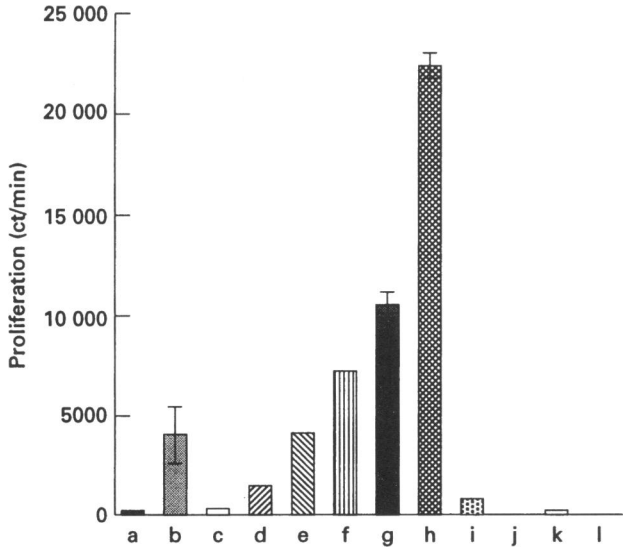

Fig. 3. Reactivity of the M.R. T cell line to various microsomal preparations from human and rat tissues. Proliferative responses in the presence of (a) complete medium, (b) RIN microsomes, (c) rat anterior pituitary, (d) rat adrenal, (e) rat hypothalamus, (f) rat cerebrum, (g) rat cerebellum, (h) rat brain cortex, (i) rat kidney, (j) rat stomach, (k) human thyroid, and (l) human liver. All microsomes were tested at 30μg/ml.

Elucidation of the molecular weight of RIN microsomal membrane proteins recognized by the M.R. and H.S. T cell lines

In order to ascertain the molecular weight of the antigens recognized by the T cell lines, the RIN microsomal membrane proteins were fractionated by SDS–PAGE and the gel cut transversally into 0·5 cm gel strips. Proteins from 15 gel strips were eluted and tested for proliferative activity of the two T cell lines. The M.R. T cell line showed proliferative activity to proteins within molecular weights of 41–47 and 72–92 kD (Fig. 5a). In contrast the H.S. T cell line showed proliferative responses to a broad range of molecular weight proteins, with significant responses to 32–36·5 and 47–56 kD components. Marginal responses were also observed to proteins in the 26–29, 56–72 and 72–92 molecular weight components (Fig. 5b).

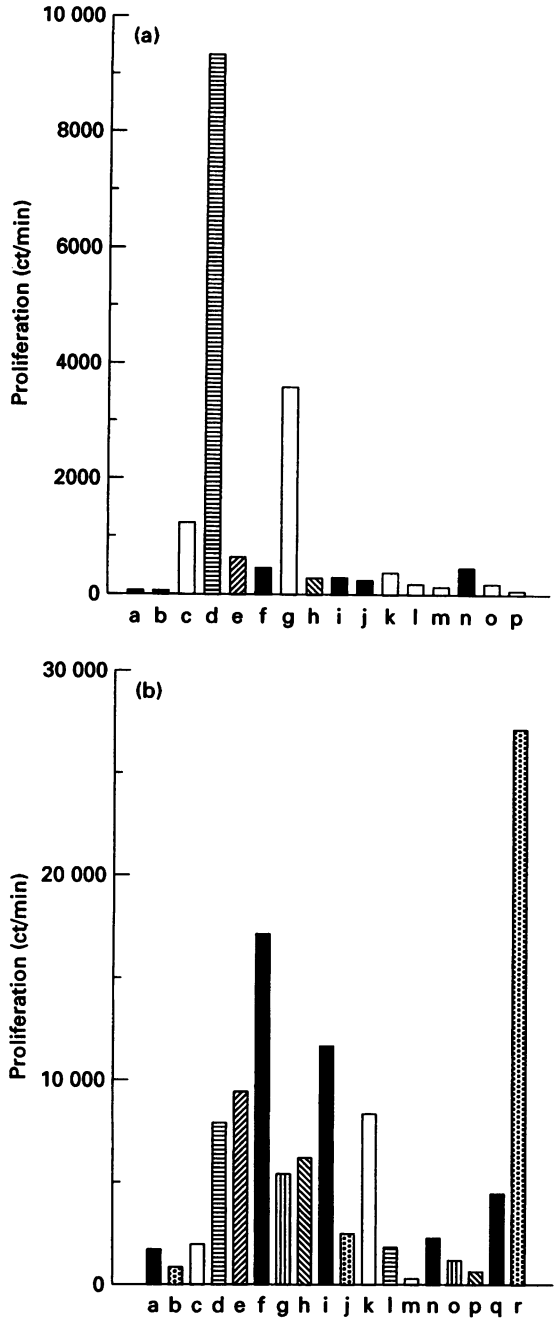

Fig. 5. Proliferative responses of (a) M.R. and (b) H.S. T cell lines to 15 different electroeluted fractions (lanes a to o) of RIN microsomes fractionated by SDS polyacrylamide gel electrophoresis.The molecular weights of the eluted fractions are as follows: lane a, > 170 kD; lane b, 120 to 170 kD; lane c, 92 to 120 kD; lane d, 72 to 92 kD; lane e, 56 to 72 kD; lane f, 47 to 56 kD; lane g, 41 to 47 kD; lane h, 36·5 to 41 kD; lane i, 32 to 36·5 kD; lane j, 29 to 32 kD; lane k, 26 to 29 kD; lane l, 23 to 26 kD; lane m, 18 to 23 kD; lane n, 14 to 18 kD and lane o, dyefront. All eluted fractions were tested at 10 μg/ml. As controls, the response to the electrophoresis buffer was also measured (lane p). For the H.S. T cell line, the proliferative response to complete medium (lane q) and RIN microsomes (30 μg/ml) is also shown (lane r).

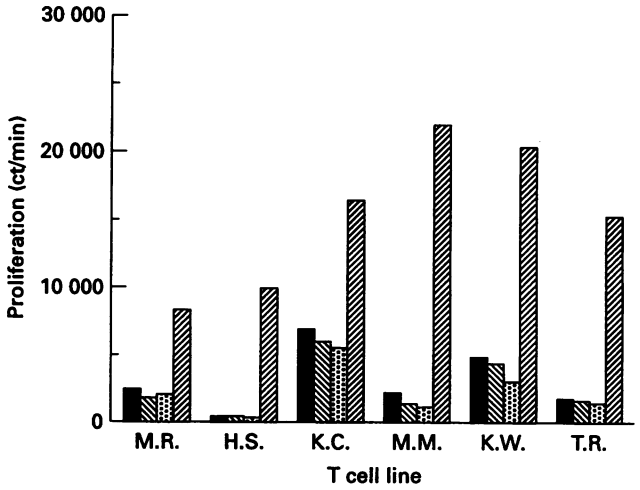

Fig. 6. Proliferative responses of the six RIN antigen specific T cell lines to purified porcine brain GAD. ^{3}H-thymidine incorporation in complete medium (■), 3 μg/ml GAD (▧), and 10 μg/ml GAD (▨) are shown. The viability of the T cell lines was assessed by measuring the proliferative response to added exogenous IL-2 (▨).

Reactivity to purified pig brain GAD

Examination of proliferative responses of the T cell lines to GAD showed that none of the six lines responded to GAD at the two concentrations of 3 and 10 μg/ml tested (Fig. 6). In contrast, all the T cell lines proliferated in response to IL-2 added to the cultures.

DISCUSSION

The reason for selecting microsomal membranes secretory granule components from the transplantable rat RIN insulinoma as a source of antigens to study T cells in human type 1 diabetic patients was based upon our previous observations in NOD mice that this preparation contains disease-related antigens important in the T cell mediated destruction of islet beta cells [10]. We used rat insulinoma tissue to study T cell reactivity in humans since large amounts of rat insulinoma tissue were available, in which secretory granules and different membrane fractions have been well characterized [18]. Rat insulinoma tissue has previously been used for growing human T cells from IDDM patients [13,14], giving compelling evidence on the cross-reactivity of islet cell proteins between human and rat tissues [8].

Islet reactive T cells are present in the peripheral blood of IDDM patients [13,14,22]. We focused our studies on newly diagnosed type 1 diabetic patients, on the basis that at this stage of the disease, T cells reacting to islet beta cell antigens implicated in ongoing beta cell destruction will be present. PBL-derived T cells from all six newly diagnosed IDDM patients responded to the RIN antigen in the presence of autologous APC. Further examination of T cell lines from two different patients showed that both the T cell lines responded vigourously to normal rat islet microsomal and cytosolic proteins, showing that the antigens recognized were present in normal islet cells and thus unlikely to be tumour specific proteins. The RIN antigen specific T cells were unlikely

to be responding to allogeneic determinants in the rat tissues since rat kidney or rat stomach microsomes did not induce proliferation of the T cells. Furthermore, microsomes from other rat or hamster insulinoma tissue grown in tissue culture did not lead to stimulation of the T cell lines (unpublished observations). There are known to be differences in the various subclones of RIN insulinoma grown in tissue culture, and indeed variations in culture conditions such as the source of fetal calf serum and addition of growth factors are known to influence the levels of certain proteins expressed by the RIN cell lines [23,24]. It is therefore possible that these subtle differences in protein expression are the reason for the lack of stimulatory activity of the tissue-culture-grown RIN cell microsomes on the T cell lines described.

Examination of microsomal membranes prepared from other rat endocrine and non-endocrine tissues for their ability to stimulate the T cell lines showed that microsomal tissue derived from different regions of the brain such as cerebrum, cerebellum and cortex contained antigen(s) recognized by the RIN insulinoma specific T cells. The cross-reactivity of the antigens recognized by T cells between islet and brain tissue may be due to the common embryonic origin of some cells within the two tissues. It is interesting that a number of the known islet beta cell autoantigens, such as insulin, GAD, ICA69 and peripherin which are recognized by autoantibodies from type 1 diabetic patients, are also shared with neural tissue.

Both the insulinoma antigen specific T cell lines examined were restricted by HLA-DR. Studies to define the precise DR restriction genes in the two patients using homologous EBV typing cells could not be performed since the EBV-transformed autologous B cells fail to process and present the RIN microsomal membrane antigens to the T cell lines (unpublished observations). There is a strong genetic susceptibility to the development or resistance of IDDM with certain MHC class II genes of HLA-DR and HLA-DQ genotypes [25]. Although the strongest susceptibilty in IDDM is with HLA-DQ genes, others have suggested a significant contribution from the HLA-DR locus to disease susceptibility [26,27].

Examination of the RIN microsomal membranes by SDS–PAGE reveals the presence of a large number of protein staining bands (>30 bands), where the RIN membranes are known to include beta cell proteins such as insulin, pro-insulin and GAD [10]. Although rodent islets utilize GAD67 predominantly [28], we do not know whether both the GAD65 and GAD67 isoforms are present in the RIN insulinoma extract used in these studies. The nature of the majority of proteins in the RIN microsomal fraction is not known, but clearly some of them may represent novel islet cell autoantigens important in T cell mediated destruction of beta cells in type 1 diabetes in the NOD mouse [10]. At this stage of work on antigen characterization, we approached studies on biochemical properties of the antigens recognized by the T cell lines using (i) electroeluted proteins from SDS-polyacrylamide gel fractionated RIN microsomes and (ii) purified autoantigens such as GAD.The molecular weights of the electroeluted proteins which induced stimulation of the antigen specific T cell lines were different in the two patients examined. While it has to be recognized that the majority of the electroeluted fractions are likely to contain different size proteins, some fractions may contain degradation products of a higher molecular weight antigen which may not be distinguished by the T cell lines. The localization of strong proliferative activity in the 41–47 and 72–92 kD for the M.R. line and that of the H.S. line to 32–36·5 and 47–56 kD, if mediated by undegraded proteins, would indicate the presence of a minimum of four different RIN microsomal antigens responsible for this activity. None of the electroeluted fractions containing proteins in the 64 kD region induced proliferation of the M.R. or H.S. T cell lines. Using purified pig brain GAD (which comigrates in the 64 kD region), which can support proliferation and growth of T cells from type 1 diabetic patients [20], we showed that GAD was not one of the proteins responsible for the stimulatory activity of the insulinoma specific T cell lines. The amino acid sequence of porcine GAD65 is not known; however, human, rat and mouse GAD65 show >95% identity in their amino acid sequence [29], making it unlikely that differences between the rat insulinoma GAD and porcine GAD are responsible for the lack of proliferation with porcine GAD. Although the RIN microsomes contain GAD, there are a number of reasons for the lack of T cell reactivity to GAD in all the T cell lines examined: (i) antigenic competition between the large number of RIN microsomal proteins may have prevented adequate processing or presentation of GAD to the putative GAD reactive T cells; (ii) the concentration of GAD in the RIN microsomal membranes; or (iii) the precursor frequency of GAD reactive T cells in the patients' peripheral blood population may be below the threshold level of detection by a proliferation assay; and (iv) GAD reactive T cells may not be present in the peripheral blood at the early stages of human disease. Since in other studies, T cell reactivity to GAD has been demonstrated in IDDM patients [5], it is possible that the failure to establish GAD specific T cells may be due to antigenic competition or to the low concentration of GAD in the RIN microsomes.

A large number of islet cell antigens recognized by antibodies from IDDM patients have been identified and characterized [2]. This is in contrast to the limited number of islet cell antigens recognized by T cells from IDDM patients, which include insulin, GAD and a 38 kD secretory granule protein. Human T cells from type 1 diabetic patients respond to porcine islet tissue [22] as well as to membranes prepared from insulinoma tissue [13,14]. As far as T cell reactivity to insulinoma tissue from normal individuals is concerned, we and others [13,14] have found the establishment of antigen specific T cell lines from normal, healthy controls to be difficult with insulinoma membranes. These findings are interesting in light of the fact that autoreactive T cells are present in normal healthy controls [21,30–32]. The reason for lack of T cell reactivity in normal individuals to insulinoma tissue is not clear, but may be related to low precursor frequency or processing events related to complex mixtures of proteins that may not generate the optimal concentration of peptides for T cell activation.

The establishment of antigen specific T cell lines to insulinoma tissue from type 1 diabetic patients will allow studies on the characterization of the insulinoma antigen(s) responsible for the induction of activation of these lines. As some of the insulinoma antigens recognized by the T cell lines cross-react with brain antigens, the use of subtraction libraries will facilitate the identification of the antigens recognized by the antigen specific T cell lines described herein.

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